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Anchoring Local Translation in Neurons

Kelsey C. Martin[1,2,*]
[1]Department of Biological Chemistry
[2]Department of Psychiatry and Biobehavioral Sciences
University of California, Los Angeles, BSRB 390B, 615 Charles E. Young Drive South, Los Angeles, CA 90095-1737, USA
*Correspondence: kcmartin@mednet.ucla.edu
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In probing how protein synthesis in neurons is coupled to extracellular stimuli, Tcherkezian et al. (2010) discover that the receptor protein DCC anchors components of the translation machinery at the plasma membrane. Binding of DCC to its ligand, netrin, triggers the release of these components to initiate spatially restricted protein synthesis.

The wiring and rewiring of neural circuits involves changes in gene expression that are both spatially and temporally regulated within individual neurons. The polarized morphology of neurons, with processes extending distances that can exceed the diameter of the cell body by orders of magnitude, poses a particular challenge to the spatial regulation of gene expression. How can localized stimuli—such as those experienced by axonal growth cones as they navigate toward their targets, or by synapses during neurotransmission—trigger rapid, compartmentalized changes in gene expression?

The discovery of local translation in axonal growth cones and dendritic spines (Sutton and Schuman 2006, Yoon et al., 2009) provides a solution to this problem by decentralizing gene expression from the neuronal nucleus and soma to the growth cone and synapse. This solution, however, raises the question of how extracellular stimuli are transduced to produce changes in protein synthesis that are spatially restricted. In their report in this issue, Flanagan and colleagues (Tcherkezian et al., 2010) provide a compelling answer: DCC, the receptor for the axonal guidance factor netrin, anchors components of the protein synthetic machinery within discrete subcellular compartments to form what the authors term a "transmembrane translational regulation complex." Binding of netrin to DCC regulates the association to the translation machinery such that extracellular signals are directly coupled to changes in protein synthesis. In this manner, netrin—initially identified as a diffusible guidance factor for long-distance axonal growth cone navigation (Kennedy et al., 1994)—plays a second, complementary role in brain wiring by coordinating local translation within neuronal subcellular compartments as they encounter new environments and stimuli.

The Flanagan group hypothesized that the local regulation of protein synthesis might involve the formation of a complex between a transmembrane protein and the translational machinery. They focused their attention on DCC because it is localized in axons and dendrites and is a receptor for netrin (Moore et al., 2007), which has been shown to mediate both axon guidance and local translation (Yoon et al., 2009). As an initial test of their hypothesis, the investigators asked whether DCC colocalizes with protein synthetic machinery. Immunocytochemical experiments at both the light and electron microscope levels reveal colocalization of DCC with ribosomal proteins and translation initiation factors at the tips of filopodia in axonal growth cones and in dendrites in puncta that are immunopositive for postsynaptic density protein 95 (PSD-95).

Coimmunoprecipitation and mass spectrometry show that DCC physically interacts with ribosomal proteins and translation initiation factors. These interactions depend on the presence of the cytoplasmic tail of DCC, and specifically on the P1 domain—a region that is conserved from nematodes to mammals. The P1 domain in turn binds directly to the ribosomal protein L5. As yet another indication of an interaction between DCC and translational machinery, in sucrose gradient sedimentation experi-

ments, DCC cosediments with small and large ribosomal subunits and with monosomes, which are usually considered to be translationally-inactive.

DCC is present in the 40S, 60S, and monosome fractions, but not in the polysome fraction, which suggests that the interaction sequesters a pool of inactive ribosomal subunits, raising the possibility that stimulation might trigger release of these subunits and their incorporation into translationally active polyribosomes. In support of this notion, addition of netrin leads to dissociation of DCC from ribosomal markers and promotes cap-dependent translation of a luciferase reporter. Further, addition of the cytoplasmic tail of DCC to cell-free reticulocyte lysates inhibits translation in a dose-dependent manner. This effect is reversed by addition of recombinant L5 protein, demonstrating a functional interaction between L5 and DCC.

To test their hypothesis in a more in vivo context, the authors ask whether the interaction between DCC and the translational machinery is required for axon guidance. To do so, they express DCC lacking the ribosome-interacting P1 domain in chick spinal cord explants, a preparation in which DCC promotes the growth of axons of commissural neurons toward the floor plate, a source of netrin. In this assay, axons from neurons expressing wild-type DCC reach the floor plate, whereas when the P1 domain is absent many do not, providing evidence that proper axon guidance depends on the association between DCC and ribosomal subunits. To directly test whether DCC regulates local translation within neurons, the authors label cultured commissural neurons with azidohomoalanine and Cy3-puromycin, reagents that permit in situ visualization of newly synthesized proteins. They find that DCC immunoreactivity overlaps with sites of new protein synthesis and that incubation with netrin increases the amount of localized new translation at puncta immunopositive for DCC.

Together, these experiments support a model in which DCC tethers ribosomal subunits, monosomes, and translation initiation factors at the membrane, with binding of netrin to DCC triggering release of ribosomes and translation factors, allowing their incorporation into translationally active polyribosomes (Figure 1). This mechanism sheds light on a number of earlier studies of local translational regulation in neurons. In one such study, Ostroff et al. (2002) used serial section electron microscopy to show that the percentage of dendritic spines in rat hippocampal CA1 neurons containing clusters of polyribosomes at their base triples after induction of long-term potentiation (LTP) of CA3-CA1 synapses. These authors hypothesized that the localized polyribosomes represent a shift of ribosomes from the dendritic shaft to the spine. However, the results of Tcherkezian et al. suggest the appealing possibility that stimuli that induce LTP trigger the release of ribosomal subunits from a transmembrane translational regulation complex, directly linking synaptic stimulation to synapse-specific activation of protein synthesis. Other studies using fluorescent reporters point to the existence of "hot spots" of local translation, which are observed even when stimuli are applied uniformly throughout the culture medium (Aakalu et al., 2001; Wang et al., 2009). These findings might now be explained by the restriction of translation to sites where components of the translational machinery are tethered to localized membrane proteins. In a recent study using reporters to visualize translation at sensory-motor synapses of the sea slug *Aplysia*, Wang et al. (2009) find that translation is regulated in the presynaptic compartment of sensory neurons in a manner that requires a calcium-dependent *trans*-synaptic signal from the motor neuron. The transmembrane translational regulation com-

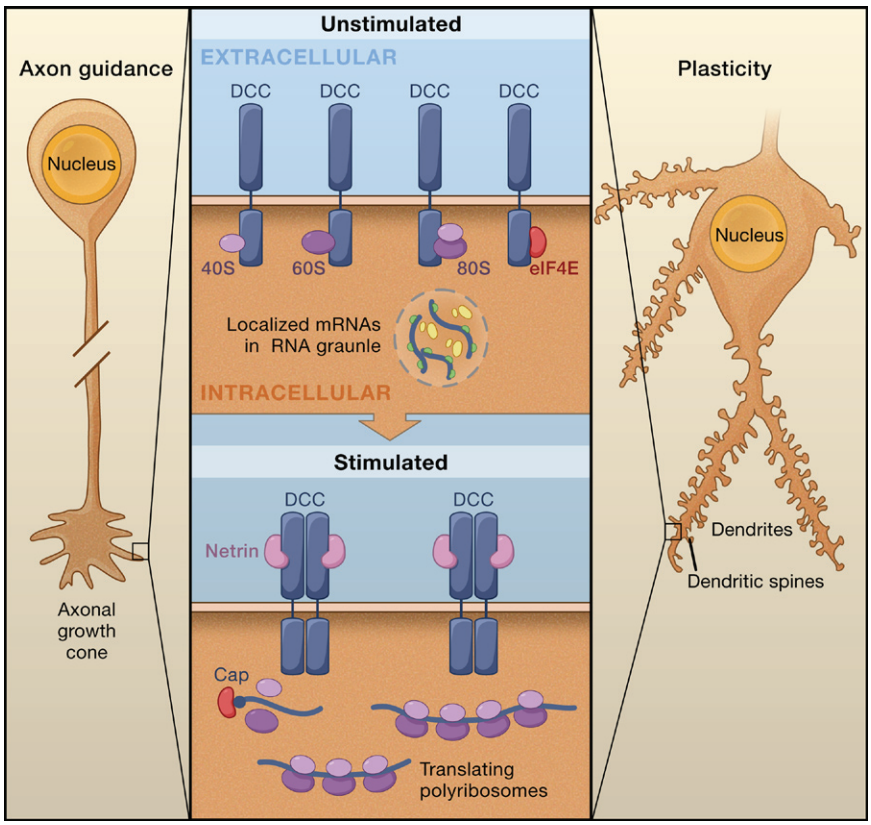

Figure 1. Coupling DCC with the Protein Synthetic Machinery

DCC interacts with protein synthetic machinery to form a transmembrane complex that regulates translation. DCC tethers the 40S and 60S ribosomal subunits, 80S monosomes, and translation initiation factors, such as eukaryotic translation initiation factor 4E (eIF4E), at the plasma membrane of axonal growth cones and, potentially, dendritic spines. In unstimulated neurons, this interaction sequesters the translational machinery. Similarly, localized mRNAs are sequestered in a dormant state, associated with RNA-binding proteins in RNA granules. When netrin binds to DCC, during axonal growth cone navigation, or perhaps during synaptic plasticity, the ribosomal components and initiation factors are released from the dimerized, active receptor and are recruited into polyribosomes that translate localized transcripts.

plex reported by Tcherkezian et al. could explain this finding, with a retrograde signal from the motor neuron triggering release and activation of the protein synthetic machinery within the presynaptic terminal.

Although the interaction between DCC and ribosomal components provides a solution to the spatial specificity of local translation, it does not address the question of transcript specificity. Studies suggest that hundreds of mRNAs may localize to particular neuronal compartments and that distinct stimuli may regulate translation of subsets of transcripts (Sutton and Schuman 2006, Wang et al., 2010). This layer of specificity may be mediated by the differential delivery of mRNAs to distinct sites within neurons, or by transcript-specific forms of translational regulation such as those mediated by microRNAs.

The discovery of a macromolecular complex that regulates translation is reminiscent of kinase-anchoring proteins that localize and synchronize signaling within the cell (Smith et al., 2006). In the classic example, A-kinase-anchoring proteins, or AKAPs, localize protein kinase A and phosphodiesterases within discrete subcellular compartments (such as the nuclear membrane, centrosome, or Golgi). This anchoring complex not only localizes the activation of protein kinase A in response to elevated cyclic AMP, but also limits the duration of signaling through the activity of the associated phosphodiesterase. In a similar manner, DCC may spatially restrict the translational response to netrin signaling and limit its duration by providing a competing binding site for ribosomes and translation factors. Further, just as an increasing number of differentially localized kinase-anchoring proteins have been discovered, DCC may represent just one of many membrane proteins that function to locally restrict translation within distinct subcellular compartments in response to specific stimuli. Endowing netrin with the capacity to regulate both axon guidance and translation within the many subcellular compartments of individual neurons provides an elegant and economical way of temporally and spatially regulating gene expression during neural circuit formation and plasticity.

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The Chemical MUPpeteer

Ivan Rodriguez[1,*]

[1]National Research Center Frontiers in Genetics, Department of Zoology and Animal Biology, University of Geneva, Geneva, Switzerland

*Correspondence: ivan.rodriguez@unige.ch

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Rodents exhibit an innate fear-like behavior when they sense the chemical traces of predators. In this issue, Papes et al. (2010) report that the major urinary proteins (Mups) released by predators are detected by sensory neurons in the mouse vomeronasal organ (which also detects pheromones involved in aggression), triggering a fear response.

To face a hostile world, animals have developed a complex array of sensory systems. In vertebrates, this sensory toolbox provides such a keen selective advantage that a considerable fraction of the vertebrate genome is devoted to its development and function. The primary sensors are specialized cells, often neurons, that are typically located in the nose and mouth. These sensors feed into parallel neural circuits that, when activated, trigger either learned responses or innate behaviors. In mammals, very little is known about the nature of the molecules responsible for activating these predetermined circuits. This situation is paralleled by our limited understanding of the hardwired circuits themselves. In this issue of *Cell*, Papes et al. (2010) unveil the molecular identity of olfactory signals called Mups (major urinary proteins) released by predators, such as cats and rats, that trigger a stereotyped and innate avoidance response in mice (Figure 1). The authors then identify sensory neurons in the mouse vomeronasal organ, a neural substructure in the nose, as sites for the detection and processing of these fear-evoking molecules.

Interspecies chemical eavesdropping is rampant in the animal kingdom, from insects to mammals. When the recipient benefits from the signal, the molecules involved are called kairomones. The Papes